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FURTHER OBSERVATIONS ON THE INFLUENCE OF INCUBATION ON THE WASSERMANN REACTION

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In a previous report¹ it was shown that complement was bound better at a temperature of about 10 C. than at 37 C. It was shown that complement binding progressed slowly so that many hours are required for its completion. At that time the optimum temperature for complement binding and the time necessary for its completion had not been determined and will be dealt with at this time.

In the present report the following points are briefly considered.

1. Incubation at 15 C., 5 C., and 2 C. for 5 hours, 10 hours and 15 hours compared with incubation at 10 C. for 5 hours, 10 hours and 15 hours.
2. Incubation at 1 C., 2 C., 3 C., 4 C., 6 C., 7 C., 8 C. and 9 C. for 5 hours and 10 hours compared with incubation at 5 C. for 5 hours and 10 hours.
3. Incubation at 3 C., 1 C. and 0.5 C. for 5 hours and for 10 hours compared with incubation at 2 C. for 5 hours and for 10 hours.
4. Incubation at 0.5 C. for 5 hours and for 10 hours compared with incubation at 1 C. for 5 hours and for 10 hours.
5. Fifteen-hour incubation at 1 C. compared with 24-hour incubation at 1 C.
6. Fifteen-hour incubation at 1 C. compared with 10-hour incubation at 1 C. Normal serum.
7. Acetone insoluble antigen compared with alcoholic extract, incubation at 1 C. and 37 C. for 1 hour and for 10 hours.
8. Alcoholic extract of beef heart compared with alcoholic extract of human heart, incubation at 1 C. and 37 C. for 1 hour and for 10 hours.
9. Acetone insoluble antigen of beef heart compared with alcoholic extract of human heart, incubation at 1 C. and at 37 C. for 1 hour and for 10 hours.
10. Nine and one-half hour incubation at 1 C. + one-half hour incubation at 37 C. compared with 10-hour incubation at 1 C.

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¹ Jour. Infect. Dis., 1918, 23, p. 173.

TEST 1

Serums 1 to 18, inclusive, incubation at 15 C., 5 C. and 2 C. for 5 hours, 10 hours and 15 hours compared with incubation at 10 C. for 5 hours, 10 hours and 15 hours. The technic of all tests in this report was identical with the technic described in the previous reports.

TABLE 1
INCUBATION AT 15 C., 5 C. AND 2 C. COMPARED WITH INCUBATION AT 10 C.

Number of Serum	Incubation Tempera- ture, C.	Incubation Time, Hours	Readings*						Results	
			Antigen Tubes			Control Tubes				
			1	2	3	1'	2'	3'		
1	10	5	+	+	0	+	+	±	Moderately positive, 2+	
	15	5	+	+	0	+	+	±	Moderately positive, 2+	
	10	10	+	±	0	+	+	±	Strongly positive, 3+	
	15	10	+	+	0	+	+	±	Moderately positive, 2+	
	10	15	+	±	0	+	+	±	Strongly positive, 3+	
	15	15	+	±	0	+	+	±	Strongly positive, 3+	
3	10	5	+	tr	0	+	+	+	Strongly positive, 4+	
	15	5	+	±	0	+	+	+	Strongly positive, 3+	
	10	10	+	tr	0	+	+	+	Strongly positive, 4+	
	15	10	+	±	0	+	+	+	Strongly positive, 3+	
	10	15	+	0	0	+	+	+	Strongly positive, 5+	
	15	15	+	±	0	+	+	+	Strongly positive, 3+	
8	10	5	+	±	0	+	+	+	Strongly positive, 3+	
	15	5	+	+	0	+	+	+	Moderately positive, 2+	
	10	10	+	tr	0	+	+	+	Strongly positive, 4+	
	15	10	+	±	0	+	+	+	Strongly positive, 3+	
	10	15	+	0	0	+	+	+	Strongly positive, 5+	
	15	15	+	±	0	+	+	+	Strongly positive, 3+	
4	10	5	+	0	0	+	+	+	Strongly positive, 5+	
	15	5	+	tr	0	+	+	+	Strongly positive, 4+	
	10	10	+	0	0	+	+	+	Strongly positive, 6+	
	15	10	+	tr	0	+	+	+	Strongly positive, 4+	
	10	15	tr	0	0	+	+	+	Strongly positive, 8+	
	15	15	+	0	0	+	+	+	Strongly positive, 5+	
5	10	5	+	tr	0	+	+	+	Strongly positive, 4+	
	15	5	+	+	0	+	+	+	Moderately positive, 2+	
	10	10	+	0	0	+	+	+	Strongly positive, 5+	
	15	10	+	±	0	+	+	+	Strongly positive, 3+	
	10	15	+	0	0	+	+	+	Strongly positive, 5+	
	15	15	+	±	0	+	+	+	Strongly positive, 3+	
6	10	5	+	0	0	+	+	+	Strongly positive, 5+	
	15	5	+	+	0	+	+	+	Moderately positive, 2+	
	10	10	+	0	0	+	+	+	Strongly positive, 6+	
	15	10	+	±	0	+	+	+	Strongly positive, 3+	
	10	15	+	0	0	+	+	+	Strongly positive, 6+	
	15	15	+	tr	0	+	+	+	Strongly positive, 4+	
7	10	5	+	0	0	+	+	+	Strongly positive, 6+	
	5	5	±?	0	0	+	+	+	Strongly positive, 7+	
	10	10	+	0	0	+	+	+	Strongly positive, 6+	
	5	10	0	0	0	+	+	+	Strongly positive, 10+	
	10	15	+	0	0	+	+	+	Strongly positive, 6+	
	5	15	0	0	0	+	+	+	Strongly positive, 10+	
8	10	5	+	0	0	+	+	+	Strongly positive, 5+	
	5	5	±	0	0	+	+	+	Strongly positive, 6+	
	10	10	+	0	0	+	+	+	Strongly positive, 5+	
	5	10	tr	0	0	+	+	+	Strongly positive, 8+	
	10	15	+	0	0	+	+	+	Strongly positive, 5+	
	5	15	0	0	0	+	+	tr	Strongly positive, 8+	

* Explanation: In all tables + means complete hemolysis; ±, hemolysis between 50% and 100%; tr (trace), hemolysis up to 50%; 0, no hemolysis.

TABLE 1—Continued
INCUBATION AT 15 C., 5 C. AND 2 C. COMPARED WITH INCUBATION AT 10 C.

Number of Serum	Incubation Tempera- ture, C.	Incubation Time, Hours	Readings							Results	
			Antigen Tubes			Control Tubes					
			1	2	3	1'	2'	3'			
9	10	5	±	0	0	+	+	±		Strongly positive, 6+	
	5	5	±?	0	0	+	+	±		Strongly positive, 7+	
	10	10	±	0	0	+	+	±		Strongly positive, 6+	
	5	10	±	0	0	+	+	±		Strongly positive, 10+	
	10	15	±	0	0	+	+	±		Strongly positive, 6+	
	5	15	±	0	0	+	+	±		Strongly positive, 10+	
10	10	5	+	tr	0	0	+	+	±	Strongly positive, 4+	
	5	5	+	tr	0	0	+	+	±	Strongly positive, 4+	
	10	10	+	0	0	0	+	+	±	Strongly positive, 5+	
	5	10	±	0	0	0	+	+	±	Strongly positive, 6+	
	10	15	+	0	0	0	+	+	±	Strongly positive, 6+	
	5	15	tr	0	0	0	+	+	±	Strongly positive, 8+	
11	10	5	+	0	0	0	+	+	±	Strongly positive, 5+	
	5	5	+	0	0	0	+	+	±	Strongly positive, 5+	
	10	10	+	0	0	0	+	+	±	Strongly positive, 5+	
	5	10	±	0	0	0	+	+	±	Strongly positive, 6+	
	10	15	+	0	0	0	+	+	±	Strongly positive, 5+	
	5	15	tr	0	0	0	+	+	±	Strongly positive, 6+	
12	10	5	+	tr	0	0	0	+	+	Strongly positive, 4+	
	5	5	+	tr	0	0	0	+	+	Strongly positive, 5+	
	10	10	+	tr	0	0	0	+	+	Strongly positive, 4+	
	5	10	±	0	0	0	+	+	±	Strongly positive, 6+	
	10	15	+	0	0	0	+	+	tr	Strongly positive, 4+	
	5	15	tr	0	0	0	+	+	tr	Strongly positive, 6+	
13	10	5	+	±	0	0	0	+	+	Strongly positive, 3+	
	2	5	+	±	0	0	0	+	+	Strongly positive, 3+	
	10	10	+	tr	0	0	0	+	+	Strongly positive, 4+	
	2	10	+	tr	0	0	0	+	+	Strongly positive, 4+	
	10	15	+	tr	0	0	0	+	+	Strongly positive, 4+	
	2	15	+	tr	0	0	0	+	+	Strongly positive, 4+	
14	10	5	+	tr	0	0	0	+	+	Strongly positive, 4+	
	2	5	±	0	0	0	0	+	+	Strongly positive, 6+	
	10	10	+	tr	0	0	0	+	+	Strongly positive, 4+	
	2	10	tr	0	0	0	0	+	+	Strongly positive, 8+	
	10	15	+	tr	0	0	0	+	+	Strongly positive, 4+	
	2	15	tr	0	0	0	0	+	+	Strongly positive, 8+	
15	10	5	+	tr	0	0	0	+	+	Strongly positive, 4+	
	2	5	+	tr	0	0	0	+	+	Strongly positive, 5+	
	10	10	+	tr	0	0	0	+	+	Strongly positive, 4+	
	2	10	+	tr	0	0	0	+	+	Strongly positive, 5+	
	10	15	+	tr	0	0	0	+	+	Strongly positive, 4+	
	2	15	±	0	0	0	0	+	+	Strongly positive, 6+	
16	10	5	±	tr	0	0	0	0	+	Strongly positive, 6+	
	2	5	tr	0	0	0	0	+	+	Strongly positive, 8+	
	10	10	±	0	0	0	0	+	+	Strongly positive, 6+	
	2	10	0	0	0	0	0	+	+	Strongly positive, 10+	
	10	15	±	0	0	0	0	+	+	Strongly positive, 6+	
	2	15	0	0	0	0	0	+	+	Strongly positive, 10+	
17	10	5	+	tr	0	0	0	0	+	Strongly positive, 4+	
	2	5	+	tr	0	0	0	0	+	Strongly positive, 5+	
	10	10	+	tr	0	0	0	0	+	Strongly positive, 5+	
	2	10	+	tr	0	0	0	0	+	Strongly positive, 6+	
	10	15	+	tr	0	0	0	0	+	Strongly positive, 5+	
	2	15	±	0	0	0	0	+	+	Strongly positive, 6+	
18	10	5	+	0	0	0	0	+	+	Strongly positive, 5+	
	2	5	tr	0	0	0	0	+	+	Strongly positive, 8+	
	10	10	±	0	0	0	0	+	+	Strongly positive, 6+	
	2	10	0	0	0	0	0	+	+	Strongly positive, 10+	
	10	15	±	0	0	0	0	+	+	Strongly positive, 6+	
	2	15	0	0	0	0	0	+	+	Strongly positive, 10+	

Table 1 shows the results obtained by comparing incubation at 15 C., at 5 C. and at 2 C. for 5 hours, for 10 hours and for 15 hours with incubation at 10 C. for 5, 10 and 15 hours. Incubation at 10 C. gave stronger positive results than did incubation at 15 C., and incubation at 5 C. and at 2 C. gave stronger positive results than did incubation at 10 C. Incubation for 10 hours gave stronger positive results than did incubation for 5 hours, and incubation for 15 hours gave stronger positive results than did incubation for 10 hours.

TEST 2

Serums 19 to 66, incubation at 1 C., 2 C., 3 C., 4 C., 6 C., 7 C., 8 C. and 9 C. for 5 hours and 10 hours was compared with incubation at 5 C. for 5 hours and 10 hours.

TABLE 2
INCUBATION AT 1 C., 2 C., 3 C., 4 C., 6 C., 7 C., 8 C. AND 9 C. COMPARED WITH
INCUBATION AT 5 C.

Number of Serum	Incubation Tempera- ture, C.	Incubation Time, Hours	Readings						Results	
			Antigen Tubes			Control Tubes				
			1	2	3	1'	2'	3'		
19	5	5	+	+	0	+	+	±	Moderately positive, 2+	
	1	5	+	+	0	+	+	±	Moderately positive, 2+	
	5	10	+	+	0	+	+	±	Moderately positive, 2+	
	1	10	+	±	0	+	+	±	Strongly positive, 3+	
20	5	5	+	±	0	+	+	±	Strongly positive, 3+	
	1	5	+	tr	0	+	+	±	Strongly positive, 4+	
	5	10	+	tr	0	+	+	±	Strongly positive, 4+	
	1	10	+	0	0	+	+	±	Strongly positive, 5+	
21	5	5	+	tr	0	+	+	±	Strongly positive, 4+	
	1	5	tr	0	0	+	+	±	Strongly positive, 8+	
	5	10	tr	0	0	+	+	±	Strongly positive, 8+	
	1	10	0	0	0	+	+	±	Strongly positive, 10+	
22	5	5	+	0	0	+	+	±	Strongly positive, 5+	
	1	5	tr	0	0	+	+	±	Strongly positive, 8+	
	5	10	tr	0	0	+	+	±	Strongly positive, 8+	
	1	10	0	0	0	+	+	±	Strongly positive, 10+	
23	5	5	+	±	0	+	+	±	Strongly positive, 3+	
	1	5	+	tr	0	+	+	±	Strongly positive, 4+	
	5	10	+	0	0	+	+	tr	Strongly positive, 4+	
	1	10	tr	0	0	+	+	tr	Strongly positive, 6+	
24	5	5	+	tr	0	+	+	±	Strongly positive, 4+	
	1	5	+	0	0	+	+	±	Strongly positive, 5+	
	5	10	+	0	0	+	+	±	Strongly positive, 5+	
	1	10	0	0	0	+	+	±	Strongly positive, 10+	
25	5	5	+	±	0	+	+	±	Strongly positive, 3+	
	2	5	+	±	0	+	+	±	Strongly positive, 3+	
	5	10	+	tr	0	+	+	±	Strongly positive, 4+	
	2	10	+	tr	0	+	+	±	Strongly positive, 4+	
26	5	5	+	0	0	+	+	±	Strongly positive, 5+	
	2	5	tr	0	0	+	+	±	Strongly positive, 8+	
	5	10	tr	0	0	+	+	±	Strongly positive, 8+	
	2	10	0	0	0	+	+	±	Strongly positive, 10+	

TABLE 2—Continued
INCUBATION AT 1 C., 2 C., 3 C., 4 C., 6 C., 7 C., 8 C. AND 9 C. COMPARED WITH
INCUBATION AT 5 C.

Number of Serum	Incuba- tion Tempera- ture, C.	Incuba- tion Time, Hours	Readings						Results	
			Antigen Tubes			Control Tubes				
			1	2	3	1'	2'	3'		
27	5	5	tr	0	0	+	+	±	Strongly positive, 8+	
			0	0	0	+	+	±	Strongly positive, 10+	
			0	0	0	+	+	±	Strongly positive, 10+	
			0	0	0	+	+	±	Strongly positive, 10+	
28	5	5	+	0	0	+	+	±	Strongly positive, 5+	
			+	0	0	+	+	±	Strongly positive, 5+	
			+	0	0	+	+	±	Strongly positive, 6+	
			tr	0	0	+	+	±	Strongly positive, 8+	
29	5	5	tr	0	0	+	+	tr	Strongly positive, 6+	
			tr	0	0	+	+	±	Strongly positive, 8+	
			0	0	0	+	+	tr	Strongly positive, 8+	
			0	0	0	+	+	±	Strongly positive, 10+	
30	5	5	tr	0	0	+	+	±	Strongly positive, 8+	
			0	0	0	+	+	±	Strongly positive, 10+	
			0	0	0	+	+	±	Strongly positive, 10+	
			0	0	0	+	+	±	Strongly positive, 10+	
31	5	5	tr	0	0	+	+	±	Strongly positive, 8+	
			0	0	0	+	+	±	Strongly positive, 10+	
			0	0	0	+	+	±	Strongly positive, 10+	
			0	0	0	+	+	±	Strongly positive, 10+	
32	5	5	+	tr	0	+	+	±	Strongly positive, 4+	
			+	0	0	+	+	±	Strongly positive, 5+	
			+	0	0	+	+	±	Strongly positive, 6+	
			tr	0	0	+	+	±	Strongly positive, 8+	
33	5	5	tr	0	0	+	+	±	Strongly positive, 8+	
			0	0	0	+	+	±	Strongly positive, 10+	
			0	0	0	+	+	±	Strongly positive, 10+	
			0	0	0	+	+	±	Strongly positive, 10+	
34	5	5	±	0	0	+	+	±	Strongly positive, 6+	
			tr	0	0	+	+	±	Strongly positive, 8+	
			tr	0	0	+	+	±	Strongly positive, 8+	
			tr	0	0	+	+	±	Strongly positive, 8+	
35	5	5	+	+	0	+	+	±	Moderately positive, 2+	
			++	+	0	+	+	±	Moderately positive, 2+	
			++	±	0	+	+	±	Strongly positive, 3+	
			++	±	0	+	+	±	Strongly positive, 3+	
36	5	5	+	+	0	+	+	±	Moderately positive, 2+	
			++	+	0	+	+	±	Moderately positive, 2+	
			++	±	0	+	+	±	Strongly positive, 3+	
			++	tr	0	+	+	±	Strongly positive, 4+	
37	5	5	+	+	tr	+	+	±	Weakly positive, 1+	
			++	+	tr	+	+	±	Weakly positive, 1+	
			++	+	0	+	+	±	Moderately positive, 2+	
			++	+	0	+	+	±	Moderately positive, 2+	
38	5	5	+	+	0	+	+	±	Moderately positive, 2+	
			++	+	0	+	+	±	Moderately positive, 2+	
			++	±	0	+	+	±	Strongly positive, 3+	
			++	±	0	+	+	±	Strongly positive, 3+	
39	5	5	+	+	0	+	+	±	Moderately positive, 2+	
			++	+	0	+	+	±	Moderately positive, 2+	
			++	±	0	+	+	±	Strongly positive, 3+	
			++	tr	0	+	+	±	Strongly positive, 4+	
40	5	5	+	tr	0	+	+	±	Strongly positive, 4+	
			++	tr	0	+	+	±	Strongly positive, 4+	
			++	0	0	+	+	±	Strongly positive, 5+	
			++	0	0	+	+	±	Strongly positive, 5+	

TABLE 2—*Continued*
 INCUBATION AT 1 C., 2 C., 3 C., 4 C., 6 C., 7 C., 8 C. AND 9 C. COMPARED WITH
 INCUBATION AT 5 C.

Number of Serum	Incubation Tempera- ture, C.	Incubation Time, Hours	Readings						Results	
			Antigen Tubes			Control Tubes				
			1	2	3	1'	2'	3'		
41	5	5	+	+	0	+	+	±	Moderately positive, 2+	
	4	5	+	+	0	+	+	±	Moderately positive, 2+	
	5	10	+	+	0	+	+	±	Moderately positive, 2+	
	4	10	+	±	0	+	+	±	Strongly positive, 3+	
42	5	5	+	+	0	+	+	+	Moderately positive, 2+	
	4	5	+	±	0	+	+	±	Strongly positive, 3+	
	5	10	+	0	0	+	+	±	Strongly positive, 5+	
	4	10	+	0	0	+	+	±	Strongly positive, 5+	
43	5	5	+	+	tr	+	+	±	Weakly positive, 1+	
	6	5	+	+	tr	+	+	±	Weakly positive, 1+	
	5	10	+	+	tr	+	+	±	Weakly positive, 1+	
	6	10	+	+	tr	+	+	±	Weakly positive, 1+	
44	5	5	+	+	0	+	+	+	Moderately positive, 2+	
	6	5	+	+	0	+	+	+	Moderately positive, 2+	
	5	10	+	+	0	+	+	+	Moderately positive, 2+	
	6	10	+	+	0	+	+	+	Moderately positive, 2+	
45	5	5	+	+	0	+	+	+	Moderately positive, 2+	
	6	5	+	+	0	+	+	+	Moderately positive, 2+	
	5	10	+	+	0	+	+	+	Moderately positive, 2+	
	6	10	+	+	0	+	+	+	Moderately positive, 2+	
46	5	5	+	tr	0	+	+	+	Strongly positive, 4+	
	6	5	+	±	0	+	+	+	Strongly positive, 3+	
	5	10	+	tr	0	+	+	+	Strongly positive, 4+	
	6	10	+	tr	0	+	+	+	Strongly positive, 4+	
47	5	5	+	+	0	+	+	+	Moderately positive, 2+	
	6	5	+	+	0	+	+	+	Moderately positive, 2+	
	5	10	+	+	0	+	+	+	Moderately positive, 2+	
	6	10	+	+	0	+	+	+	Moderately positive, 2+	
48	5	5	+	±	0	+	+	+	Strongly positive, 3+	
	6	5	+	±	0	+	+	+	Strongly positive, 3+	
	5	10	+	tr	0	+	+	+	Strongly positive, 4+	
	6	10	+	tr	0	+	+	+	Strongly positive, 4+	
49	5	5	+	+	0	+	+	+	Strongly positive, 3+	
	7	5	+	±	0	+	+	+	Strongly positive, 3+	
	5	10	+	tr	0	+	+	+	Strongly positive, 4+	
	7	10	+	±	0	+	+	+	Strongly positive, 3+	
50	5	5	+	tr	0	+	+	+	Strongly positive, 4+	
	7	5	+	±	0	+	+	+	Strongly positive, 3+	
	5	10	+	0	0	+	+	+	Strongly positive, 6+	
	7	10	+	tr	0	+	+	+	Strongly positive, 4+	
51	5	5	+	+	0	+	+	+	Moderately positive, 2+	
	7	5	+	+	0	+	+	+	Moderately positive, 2+	
	5	10	+	±	0	+	+	+	Strongly positive, 3+	
	7	10	+	+	0	+	+	+	Moderately positive, 2+	
52	5	5	+	0	0	+	+	+	Strongly positive, 5+	
	7	5	+	tr	0	0	+	+	Strongly positive, 4+	
	5	10	+	0	0	+	+	+	Strongly positive, 5+	
	7	10	+	tr	0	0	+	+	Strongly positive, 4+	
53	5	5	+	tr	0	0	+	+	Strongly positive, 4+	
	7	5	+	±	0	0	+	+	Strongly positive, 4+	
	5	10	+	tr	0	0	+	+	Strongly positive, 6+	
	7	10	+	tr	0	0	+	+	Strongly positive, 4+	

TABLE 2—Continued
INCUBATION AT 1 C., 2 C., 3 C., 4 C., 6 C., 7 C., 8 C. AND 9 C. COMPARED WITH
INCUBATION AT 5 C.

Number of Serum	Incuba-tion Tempera-ture, C.	Incuba-tion Time, Hours	Readings						Results	
			Antigen Tubes			Control Tubes				
			1	2	3	1'	2'	3'		
54	5	5	+	0	0	+	+	±	Strongly positive, 5+	
	7	5	+	tr	0	+	+	+	Strongly positive, 4+	
	5	10	tr	0	0	+	+	+	Strongly positive, 8+	
	7	10	+	0	0	+	+	±	Strongly positive, 5+	
55	5	5	+	±	0	+	+	±	Strongly positive, 3+	
	8	5	+	+	0	+	+	+	Moderately positive, 2+	
	5	10	+	tr	0	+	+	+	Strongly positive, 4+	
	8	10	+	±	0	+	+	+	Strongly positive, 3+	
56	5	5	+	±	0	+	+	±	Strongly positive, 3+	
	8	5	+	+	tr	+	+	+	Weakly positive, 1+	
	5	10	+	tr	0	+	+	+	Strongly positive, 4+	
	8	10	+	+	0	+	+	±	Moderately positive, 2+	
57	5	5	+	±	0	+	+	±	Strongly positive, 3+	
	8	5	+	+	0	+	+	±	Moderately positive, 2+	
	5	10	+	tr	0	+	+	+	Strongly positive, 4+	
	8	10	+	±	0	+	+	±	Strongly positive, 3+	
58	5	5	+	±	0	+	+	±	Strongly positive, 3+	
	8	5	+	+	tr	+	+	+	Weakly positive, 1+	
	5	10	+	+	0	+	+	+	Strongly positive, 3+	
	8	10	+	+	0	+	+	±	Moderately positive, 2+	
59	5	5	+	+	0	+	+	±	Moderately positive, 2+	
	8	5	+	+	0	+	+	+	Moderately positive, 2+	
	5	10	+	±	0	+	+	+	Strongly positive, 3+	
	8	10	+	+	0	+	+	±	Moderately positive, 2+	
60	5	5	+	+	0	+	+	±	Moderately positive, 2+	
	8	5	+	+	tr	+	+	+	Weakly positive, 1+	
	5	10	+	+	0	+	+	+	Moderately positive, 2+	
	8	10	+	+	tr	+	+	+	Weakly positive, 1+	
61	5	5	+	tr	0	+	+	±	Strongly positive, 4+	
	9	5	+	±	0	+	+	+	Strongly positive, 3+	
	5	10	+	0	0	+	+	+	Strongly positive, 5+	
	9	10	+	tr	0	+	+	±	Strongly positive, 4+	
62	5	5	+	±	0	+	+	±	Strongly positive, 3+	
	9	5	+	+	0	+	+	+	Moderately positive, 2+	
	5	10	+	0	0	+	+	+	Strongly positive, 5+	
	9	10	+	±	0	+	+	±	Strongly positive, 3+	
63	5	5	+	0	0	+	+	±	Strongly positive, 5+	
	9	5	+	tr	0	+	+	+	Strongly positive, 4+	
	5	10	tr	0	0	+	+	+	Strongly positive, 8+	
	9	10	+	tr	0	+	+	±	Strongly positive, 4+	
64	5	5	+	tr	0	+	+	±	Strongly positive, 4+	
	9	5	+	±	0	+	+	+	Strongly positive, 3+	
	5	10	+	0	0	+	+	+	Strongly positive, 5+	
	9	10	+	±	0	+	+	±	Strongly positive, 3+	
65	5	5	+	±	0	+	+	±	Strongly positive, 3+	
	9	5	+	+	0	+	+	+	Strongly positive, 3+	
	5	10	+	0	0	+	+	+	Strongly positive, 5+	
	9	10	+	±	0	+	+	±	Strongly positive, 3+	
66	5	5	+	±	0	+	+	±	Strongly positive, 3+	
	9	5	+	+	0	+	+	+	Moderately positive, 2+	
	5	10	+	tr	0	+	+	+	Strongly positive, 4+	
	9	10	+	+	0	+	+	±	Moderately positive, 2+	

The results obtained by comparing incubation at 1 C., 2 C., 3 C., 4 C., 6 C., 7 C., 8 C. and 9 C. for 5 hours and 10 hours with incubation at 5 C. for 5 and 10 hours are shown in Table 2. Incubation at 1 C., 2 C., 3 C. and 4 C. gave stronger positive results than did incubation at 5 C. and incubation at 6 C., 7 C., 8 C. and 9 C. gave weaker positive results than did incubation at 5 C. Incubation for 10 hours gave stronger positive results than did incubation for 5 hours.

TEST 3

Serums 67 to 84, inclusive, incubation at 3 C., 1 C. and 0.5 C. for 5 hours and for 10 hours was compared with incubation at 2 C. for 5 and 10 hours.

TABLE 3
INCUBATION AT 3 C., 1 C. AND 0.5 C. COMPARED WITH INCUBATION AT 2 C.

Number of Serum	Incubation Tempera- ture, C.	Incubation Time, Hours	Readings						Results	
			Antigen Tubes			Control Tubes				
			1	2	3	1'	2'	3'		
67	2	5	+	0	0	+	+	±	Strongly positive, 5+	
	3	5	+	tr	0	+	+	±	Strongly positive, 4+	
	2	10	±	0	0	+	+	±	Strongly positive, 6+	
	3	10	+	0	0	+	+	±	Strongly positive, 5+	
68	2	5	+	tr	0	+	+	±	Strongly positive, 4+	
	3	5	+	±	0	+	+	±	Strongly positive, 3+	
	2	10	+	0	0	+	+	±	Strongly positive, 5+	
	3	10	+	tr	0	+	+	±	Strongly positive, 4+	
69	2	5	+	0	0	+	+	±	Strongly positive, 5+	
	3	5	+	tr	0	+	+	±	Strongly positive, 4+	
	2	10	±	0	0	+	+	±	Strongly positive, 6+	
	3	10	+	0	0	+	+	±	Strongly positive, 5+	
70	2	5	+	0	0	+	+	±	Strongly positive, 5+	
	3	5	+	tr	0	+	+	±	Strongly positive, 4+	
	2	10	±	0	0	+	+	±	Strongly positive, 6+	
	3	10	+	tr	0	+	+	±	Strongly positive, 4+	
71	2	5	+	+	0	+	+	±	Moderately positive, 2+	
	3	5	+	+	0	+	+	±	Moderately positive, 2+	
	2	10	+	±	0	+	+	±	Strongly positive, 3+	
	3	10	+	±	0	+	+	±	Strongly positive, 3+	
72	2	5	+	+	0	+	+	±	Moderately positive, 2+	
	3	5	+	+	0	+	+	±	Moderately positive, 2+	
	2	10	+	±	0	+	+	±	Strongly positive, 3+	
	3	10	+	±	0	+	+	±	Strongly positive, 3+	
73	2	5	+	+	tr	+	+	±	Weakly positive, 1+	
	1	5	+	+	tr	+	+	±	Weakly positive, 1+	
	2	10	+	+	0	+	+	±	Moderately positive, 2+	
	1	10	+	+	0	+	+	±	Moderately positive, 2+	
74	2	5	+	+	tr	+	+	±	Weakly positive, 1+	
	1	5	+	+	tr	+	+	±	Weakly positive, 1+	
	2	10	+	+	tr	+	+	±	Weakly positive, 1+	
	1	10	+	+	0	+	+	±	Moderately positive, 2+	

TABLE 3—*Continued*
INCUBATION AT 3 C., 1 C. AND 0.5 C. COMPARED WITH INCUBATION AT 2 C.

Number of Serum	Incubation Tempera- ture, C.	Incubation Time, Hours	Readings						Results	
			Antigen Tubes			Control Tubes				
			1	2	3	1'	2'	3'		
75	2	5	+	+	tr	+	+	±	Weakly positive, 1+	
	1	5	+	+	tr	+	+	±	Weakly positive, 1+	
	2	10	+	+	tr	+	+	±	Weakly positive, 1+	
	1	10	+	+	0	+	+	±	Moderately positive, 2+	
76	2	5	+	±	0	+	+	±	Strongly positive, 3+	
	1	5	+	tr	0	+	+	±	Strongly positive, 4+	
	2	10	+	tr	0	+	+	±	Strongly positive, 4+	
	1	10	+	0	+	+	+	±	Strongly positive, 5+	
77	2	5	+	+	0	+	+	±	Moderately positive, 2+	
	1	5	+	+	0	+	+	±	Moderately positive, 2+	
	2	10	+	+	0	+	+	±	Moderately positive, 2+	
	1	10	+	±	0	+	+	±	Strongly positive, 3+	
78	2	5	+	±	0	+	+	±	Strongly positive, 3+	
	1	5	+	tr	0	+	+	±	Strongly positive, 4+	
	2	10	+	0	0	+	+	±	Strongly positive, 5+	
	1	10	+	0	0	+	+	±	Strongly positive, 5+	
79	2	5	+	tr	0	+	+	±	Strongly positive, 4+	
	0.5	5	+	tr	0	+	+	±	Strongly positive, 4+	
	2	10	+	tr	0	+	+	±	Strongly positive, 4+	
	0.5	10	+	0	0	+	+	±	Strongly positive, 5+	
80	2	5	+	±	0	+	+	±	Strongly positive, 3+	
	0.5	5	+	±	0	+	+	±	Strongly positive, 3+	
	2	10	+	±	0	+	+	±	Strongly positive, 3+	
	0.5	10	+	tr	0	+	+	±	Strongly positive, 4+	
81	2	5	+	tr	0	+	+	±	Strongly positive, 4+	
	0.5	5	+	0	0	+	+	±	Strongly positive, 5+	
	2	10	+	c	0	+	+	±	Strongly positive, 5+	
	0.5	10	+	±	0	+	+	±	Strongly positive, 6+	
82	2	5	+	+	0	+	+	±	Moderately positive, 2+	
	0.5	5	+	+	0	+	+	±	Moderately positive, 2+	
	2	10	+	±	0	+	+	±	Strongly positive, 3+	
	0.5	10	+	tr	0	+	+	±	Strongly positive, 4+	
83	2	5	+	+	0	+	+	±	Moderately positive, 2+	
	0.5	5	+	+	0	+	+	±	Moderately positive, 2+	
	2	10	+	+	0	+	+	±	Moderately positive, 2+	
	0.5	10	+	±	0	+	+	±	Strongly positive, 3+	
84	2	5	+	+	tr	+	+	±	Weakly positive, 1+	
	0.5	5	+	+	tr	+	+	±	Weakly positive, 1+	
	2	10	+	+	tr	+	+	±	Weakly positive, 1+	
	0.5	10	+	+	0	+	+	±	Moderately positive, 2+	

Table 3 shows the results obtained by comparing incubation at 3 C., 1 C. and 0.5 C. for 5 hours and 10 hours with incubation at 2 C. for 5 and 10 hours. Incubation at 3 C. gave weaker positive results than did incubation at 2 C. and incubation at 1 C. and at 0.5 C. gave stronger positive results than did incubation at 2 C. Incubation for 10 hours gave stronger positive results than did incubation for 5 hours.

TEST 4

Incubation at 0.5 C. for 5 hours and for 10 hours compared with incubation at 1 C. for 5 and 10 hours on 12 serums—85 to 96, inclusive.

TAGLE 4
INCUBATION AT 0.5 C. COMPARED WITH INCUBATION AT 1 C.

Number of Serum	Incubation Tempera- ture, C.	Incubation Time, Hours	Readings						Results	
			Antigen Tubes			Control Tubes				
			1	2	3	1'	2'	3'		
85	1	5	tr	0	0	+	+	±	Strongly positive, 8+	
	0.5	5	tr	0	0	+	+	±	Strongly positive, 8+	
	1	10	0	0	0	+	+	±	Strongly positive, 10+	
	0.5	10	0	0	0	+	+	±	Strongly positive, 10+	
86	1	5	±	0	0	+	+	±	Strongly positive, 6+	
	0.5	5	±	0	0	+	+	±	Strongly positive, 6+	
	1	10	tr	0	0	+	+	±	Strongly positive, 8+	
	0.5	10	tr	0	0	+	+	±	Strongly positive, 8+	
87	1	5	tr	0	0	+	+	±	Strongly positive, 8+	
	0.5	5	tr	0	0	+	+	±	Strongly positive, 8+	
	1	10	0	0	0	+	+	±	Strongly positive, 10+	
	0.5	10	0	0	0	+	+	±	Strongly positive, 10+	
88	1	5	±	0	0	+	+	±	Strongly positive, 6+	
	0.5	5	±	0	0	+	+	±	Strongly positive, 6+	
	1	10	tr	0	0	+	+	±	Strongly positive, 8+	
	0.5	10	tr	0	0	+	+	±	Strongly positive, 8+	
89	1	5	+	+	0	+	+	±	Moderately positive, 2+	
	0.5	5	++	+	0	+	+	±	Moderately positive, 2+	
	1	10	++	±	0	+	+	±	Strongly positive, 3+	
	0.5	10	+	±	0	+	+	±	Strongly positive, 3+	
90	1	5	+	+	0	+	+	±	Moderately positive, 2+	
	0.5	5	++	+	0	+	+	±	Moderately positive, 2+	
	1	10	++	±	0	+	+	±	Strongly positive, 3+	
	0.5	10	+	±	0	+	+	±	Strongly positive, 3+	
91	1	5	+	+	0	+	+	±	Moderately positive, 2+	
	0.5	5	++	+	0	+	+	±	Moderately positive, 2+	
	1	10	++	±	0	+	+	±	Strongly positive, 3+	
	0.5	10	+	±	0	+	+	±	Strongly positive, 3+	
92	1	5	+	+	0	+	+	±	Moderately positive, 2+	
	0.5	5	++	+	0	+	+	±	Moderately positive, 2+	
	1	10	++	±	0	+	+	±	Strongly positive, 3+	
	0.5	10	+	±	0	+	+	±	Strongly positive, 3+	
93	1	5	+	+	0	+	+	±	Moderately positive, 2+	
	0.5	5	++	+	0	+	+	±	Moderately positive, 2+	
	1	10	++	tr	0	+	+	±	Strongly positive, 4+	
	0.5	10	+	tr	0	+	+	±	Strongly positive, 4+	
94	1	5	+	+	tr	+	+	±	Weakly positive, 1+	
	0.5	5	++	+	tr	+	+	±	Weakly positive, 1+	
	1	10	++	±	0	+	+	±	Strongly positive, 3+	
	0.5	10	+	±	0	+	+	±	Strongly positive, 3+	
95	1	5	+	+	tr	+	+	±	Weakly positive, 1+	
	0.5	5	++	+	tr	+	+	±	Weakly positive, 1+	
	1	10	++	0	+	+	+	+	Moderately positive, 2+	
	0.5	10	+	+	0	+	+	+	Moderately positive, 2+	
96	1	5	+	+	tr	+	+	±	Weakly positive, 1+	
	0.5	5	++	+	tr	+	+	±	Weakly positive, 1+	
	1	10	++	±	0	+	+	+	Strongly positive, 3+	
	0.5	10	+	±	0	+	+	+	Strongly positive, 3+	

Table 4 shows the results obtained by comparing incubation at 0.5 C. for 5 and 10 hours with incubation at 1 C. for 5 and 10 hours—serums 85 to 96, inclusive. The results obtained with incubation at 0.5 C. were identical with those obtained with incubation at 1 C. Ten-hour incubation at both of these temperatures gave stronger positive results than did 5-hour incubation.

TEST 5

Incubation for 15 hours at 1 C. compared with incubation for 24 hours at 1 C.—serums 97 to 120, inclusive.

TABLE 5
FIFTEEN-HOUR INCUBATION AT 1 C. COMPARED WITH TWENTY-FOUR-HOUR INCUBATION AT 1 C.

Number of Serum	Incubation Tempera- ture, C.	Incubation Time, Hours	Readings						Results	
			Antigen Tubes			Control Tubes				
			1	2	3	1'	2'	3'		
97	1	15	±	0	0	+	+	±	Strongly positive, 6+	
	1	24	tr	0	0	+	+	tr		
98	1	15	tr	0	0	+	+	±	Strongly positive, 8+	
	1	24	0	0	0	+	+	±		
99	1	15	tr	0	0	+	+	±	Strongly positive, 8+	
	1	24	0	0	0	+	+	tr		
100	1	15	tr	0	0	+	+	±	Strongly positive, 8+	
	1	24	0	0	0	+	+	±		
101	1	15	±	0	0	+	+	±	Strongly positive, 6+	
	1	24	tr	0	0	+	+	tr		
102	1	15	tr	0	0	+	+	±	Strongly positive, 8+	
	1	24	0	0	0	+	+	tr		
103	1	15	tr	0	0	+	+	±	Strongly positive, 8+	
	1	24	0	0	0	+	+	±		
104	1	15	±	0	0	+	+	±	Strongly positive, 6+	
	1	24	tr	0	0	+	+	±		
105	1	15	tr	0	0	+	+	±	Strongly positive, 8+	
	1	24	0	0	0	+	+	±		
106	1	15	±	0	0	+	+	±	Strongly positive, 6+	
	1	24	0	0	0	+	+	tr		
107	1	15	+	tr	0	+	+	±	Strongly positive, 4+	
	1	24	±	0	0	+	+	tr		
108	1	15	±	0	0	+	+	±	Strongly positive, 6+	
	1	24	0	0	0	+	+	tr		
109	1	15	tr	tr	0	+	+	±	Unfit	
	1	24	0	0	0	tr	±	tr		
110	1	15	±	tr	0	+	+	±	Unfit Strongly positive, 6+	
	1	24	tr	0	0	+	+	tr		
111	1	15	0	0	0	+	+	±	Strongly positive, 10+	
	1	24	0	0	0	+	+	±		
112	1	15	+	±	0	+	+	±	Strongly positive, 3+ Unfit	
	1	24	tr	tr	0	+	+	±		

TABLE 5—Continued
FIFTEEN-HOUR INCUBATION AT 1 C. COMPARED WITH TWENTY-FOUR-HOUR INCUBATION AT 1 C.

Number of Serum	Incubation Tempera- ture, C.	Incubation Time, Hours	Readings						Results	
			Antigen Tubes			Control Tubes				
			1	2	3	1'	2'	3'		
113	1	15	+	±	0	+	+	±	Strongly positive, 3+	
	1	24	+	tr	0	+	+	tr	Strongly positive, 3+	
114	1	15	+	±	0	+	+	±	Strongly positive, 3+	
	1	24	0	0	0	+	+	tr	Strongly positive, 8+	
115	1	15	+	±	tr	+	+	±	Unfit?	
	1	24	+	tr	tr	+	+	±	Unfit	
116	1	15	tr	0	0	+	+	±	Strongly positive, 8+	
	1	24	0	0	0	+	+	±	Unfit	
117	1	15	0	0	0	tr	±	tr	Unfit	
	1	24	0	0	0	tr	±	±	Unfit	
118	1	15	0	0	0	+	+	±	Strongly positive, 10+	
	1	24	0	0	0	+	+	tr	Strongly positive, 8+	
119	1	15	0	0	0	tr	±	±	Unfit	
	1	24	0	0	0	0	±	tr	Unfit	
120	1	15	0	0	0	+	+	tr	Strongly positive, 8+	
	1	24	0	0	0	tr	±	tr	Unfit	

The results obtained by comparing incubation for 15 hours at 1 C. with incubation for 24 hours at 1 C. are shown in Table 5. With serums 97 to 108, inclusive, the results were good, and with incubation for 24 hours the results were frequently stronger positive than with incubation for 15 hours, showing that complement binding was not always completed in 15 hours. With serums 109 to 120, inclusive, the results were poor. As these 12 serums were tested with the same lot of complement, and as such poor results were not obtained otherwise with incubation for 15 hours the complement is blamed for these poor results.

TEST 6

Fifteen-hour incubation at 1 C. was compared with 10-hour incubation at 1 C. on 24 normal human serums (121 to 144, inclusive).

TABLE 6
FIFTEEN-HOUR INCUBATION AT 1 C. COMPARED WITH TEN-HOUR INCUBATION AT 1 C.
NORMAL SERUM

Number of Serum	Incubation Tempera- ture, C.	Incubation Time, Hours	Readings						Results	
			Antigen Tubes			Control Tubes				
			1	2	3	1'	2'	3'		
121	1	10	+	+	±	+	+	±	Negative, —	
	1	15	+	+	±	+	+	±	Negative, —	
122	1	10	+	+	±	+	+	±	Negative, —	
	1	15	+	+	±	+	+	±	Negative, —	

TABLE 6—*Continued*
 FIFTEEN-HOUR INCUBATION AT 1 C. COMPARED WITH TEN-HOUR INCUBATION AT 1 C.
 NORMAL SERUM

Number of Serum	Incuba- tion Tempera- ture, C.	Incuba- tion Time, Hours	Readings						Results	
			Antigen Tubes			Control Tubes				
			1	2	3	1'	2'	3'		
123	1	10	+	+	tr	+	+	tr	Negative, —	
	1	15	+	+	tr	+	+	tr	Negative, —	
124	1	10	+	+	±	+	+	±	Negative, —	
	1	15	+	+	tr	+	+	tr	Negative, —	
125	1	10	+	+	±	+	+	±	Negative, —	
	1	15	+	+	+	+	+	±	Negative, —	
126	1	10	+	+	±	+	+	±	Negative, —	
	1	15	+	+	tr	+	+	tr	Negative, —	
127	1	10	+	+	tr	+	+	tr	Negative, —	
	1	15	+	+	tr	+	+	tr	Negative, —	
128	1	10	+	+	±	+	+	±	Negative, —	
	1	15	+	+	±	+	+	±	Negative, —	
129	1	10	+	+	±	+	+	±	Negative, —	
	1	15	+	+	±	+	+	±	Negative, —	
130	1	10	+	+	±	+	+	±	Negative, —	
	1	15	+	+	tr	+	+	tr	Negative, —	
131	1	10	+	+	tr	+	+	tr	Negative, —	
	1	15	+	+	tr	+	+	tr	Negative, —	
132	1	10	+	+	±	+	+	±	Negative, —	
	1	15	+	+	tr	+	+	tr	Negative, —	
133	1	10	+	+	±	+	+	±	Negative, —	
	1	15	+	+	±	+	+	±	Negative, —	
134	1	10	+	+	±	+	+	±	Negative, —	
	1	15	+	+	±	+	+	±	Negative, —	
135	1	10	+	+	±	+	+	±	Negative, —	
	1	15	+	+	±	+	+	±	Negative, —	
136	1	10	+	+	±	+	+	±	Negative, —	
	1	15	+	+	±	+	+	±	Negative, —	
137	1	10	+	+	±	+	+	±	Negative, —	
	1	15	+	+	±	+	+	±	Negative, —	
138	1	10	+	+	±	+	+	±	Negative, —	
	1	15	+	+	±	+	+	±	Negative, —	
139	1	10	+	+	±	+	+	±	Negative, —	
	1	15	+	+	±	+	+	±	Negative, —	
140	1	10	+	+	tr	+	+	tr	Negative, —	
	1	15	+	+	tr	+	+	tr	Negative, —	
141	1	10	+	+	±	+	+	±	Negative, —	
	1	15	+	+	±	+	+	±	Negative, —	
142	1	10	+	+	tr	+	+	tr	Negative, —	
	1	15	+	+	0	+	+	0	Negative, —	
143	1	10	+	+	tr	+	+	tr	Negative, —	
	1	15	+	+	tr	+	+	tr	Negative, —	
144	1	10	+	+	tr	+	+	tr	Negative, —	
	1	15	+	+	tr	+	+	tr	Negative, —	

Table 6 shows the results obtained by comparing 15-hour incubation at 1 C. with 10-hour incubation at 1 C. on 24 specimens of normal human serum. All of the results were negative.

TEST 7

On Serums 145 to 160, inclusive, acetone insoluble antigen of beef heart (A. I. B. H.) was compared with alcoholic extract of beef heart (A. E. B. H.). Incubation at 1 C. and at 37 C. for 1 hour and for 10 hours.

TABLE 7
ACETONE INSOLUBLE ANTIGEN COMPARED WITH CRUDE ALCOHOLIC EXTRACT, INCUBATION AT
1 C. AND 37 C. FOR 1 HOUR AND 10 HOURS

Number of Serum	Kind of Antigen	Incuba- tion Temper- ature, C.	Incuba- tion Time, Hours	Readings							Results	
				Antigen Tubes			Control Tubes					
				1	2	3	1'	2'	3'			
145	A.E.B.H.	1	1	+	+	±	+	+	±		Negative,	
	A.I.B.H.	1	1	+	+	±	+	+	±		Negative,	
	A.E.B.H.	37	1	+	+	±	+	+	±		Negative,	
	A.I.B.H.	37	1	+	+	±	+	+	±		Negative,	
	A.E.B.H.	1	10	+	+	±	+	+	±		Negative,	
	A.I.B.H.	1	10	+	+	±	+	+	±		Negative,	
	A.E.B.H.	37	10	+	+	tr	+	+	tr		Negative,	
	A.I.B.H.	37	10	+	+	tr	+	+	tr		Negative,	
146	A.E.B.H.	1	1	+	+	±	+	+	±		Negative,	
	A.I.B.H.	1	1	+	+	±	+	+	±		Negative,	
	A.E.B.H.	37	1	+	+	±	+	+	±		Negative,	
	A.I.B.H.	37	1	+	+	±	+	+	±		Negative,	
	A.E.B.H.	1	10	+	+	0	+	+	±		Strongly positive,	
	A.I.B.H.	1	10	+	+	±	+	+	±		Negative,	
	A.E.B.H.	37	10	+	+	tr	+	+	tr		Negative,	
	A.I.B.H.	37	10	+	+	tr	+	+	tr		Negative,	
147	A.E.B.H.	1	1	+	+	±	+	+	±		Negative,	
	A.I.B.H.	1	1	+	+	±	+	+	±		Negative,	
	A.E.B.H.	37	1	+	+	±	+	+	±		Negative,	
	A.I.B.H.	37	1	+	+	±	+	+	±		Negative,	
	A.E.B.H.	1	10	+	tr	0	+	+	±		Strongly positive,	
	A.I.B.H.	1	10	+	+	±	+	+	±		Negative,	
	A.E.B.H.	37	10	+	+	tr	+	+	tr		Negative,	
	A.I.B.H.	37	10	+	+	tr	+	+	tr		Negative,	
148	A.E.B.H.	1	1	+	+	tr	+	+	±		Weakly positive,	
	A.I.B.H.	1	1	+	+	±	+	+	±		Negative,	
	A.E.B.H.	37	1	+	+	tr	+	+	±		Weakly positive,	
	A.I.B.H.	37	1	+	+	tr	+	+	±		Negative,	
	A.E.B.H.	1	10	+	tr	0	+	+	±		Strongly positive,	
	A.I.B.H.	1	10	+	+	±	+	+	±		Negative,	
	A.E.B.H.	37	10	+	+	0	+	+	tr		Weakly positive,	
	A.I.B.H.	37	10	+	+	tr?	+	+	tr		Faintly positive,	
149	A.E.B.H.	1	1	+	+	tr	+	+	±		Weakly positive,	
	A.I.B.H.	1	1	+	+	±	+	+	±		Negative,	
	A.E.B.H.	37	1	+	+	±?	+	+	±		Faintly positive,	
	A.I.B.H.	37	1	+	+	±	+	+	±		Negative,	
	A.E.B.H.	1	10	+	+	0	+	+	±		Moderately positive, 2+	
	A.I.B.H.	1	10	+	+	±	+	+	±		Negative,	
	A.E.B.H.	37	10	+	+	tr?	+	+	tr		Faintly positive,	
	A.I.B.H.	37	10	+	+	tr	+	+	tr		Negative,	

TABLE 7—Continued

ACETONE INSOLUBLE ANTIGEN COMPARED WITH CRUDE ALCOHOLIC EXTRACT, INCUBATION AT
1 C. AND 37 C. FOR 1 HOUR AND 10 HOURS

Number of Serum	Kind of Antigen	Incuba- tion Temper- ature, C.	Incuba- tion Time, Hours	Readings						Results	
				Antigen Tubes			Control Tubes				
				1	2	3	1'	2'	3'		
150	A.E.B.H.	1	1	+	+	±	+	+	±	Negative,	—
	A.I.B.H.	1	1	+	+	±	+	+	±	Negative,	—
	A.E.B.H.	37	1	+	+	±	+	+	±	Negative,	—
	A.I.B.H.	37	1	+	+	±	+	+	±	Negative,	—
	A.E.B.H.	1	10	+	±	0	+	+	±	Strongly positive,	3+
	A.I.B.H.	1	10	+	+	±	+	+	±	Negative,	—
	A.E.B.H.	37	10	+	+	tr?	+	+	tr	Faintly positive,	±
	A.I.B.H.	37	10	+	+	tr	+	+	tr	Negative,	—
151	A.E.B.H.	1	1	+	+	±	+	+	±	Negative,	—
	A.I.B.H.	1	1	+	+	±	+	+	±	Negative,	—
	A.E.B.H.	37	1	+	+	±	+	+	±	Negative,	—
	A.I.B.H.	37	1	+	+	±	+	+	±	Negative,	—
	A.E.B.H.	1	10	+	+	tr	+	+	±	Weakly positive,	1+
	A.I.B.H.	1	10	+	+	±	+	+	±	Negative,	—
	A.E.B.H.	37	10	+	+	tr	+	+	tr	Negative,	—
	A.I.B.H.	37	10	+	+	tr	+	+	tr	Negative,	—
152	A.E.B.H.	1	1	+	+	±	+	+	±	Negative,	—
	A.I.B.H.	1	1	+	+	±	+	+	±	Negative,	—
	A.E.B.H.	37	1	+	+	±	+	+	±	Negative,	—
	A.I.B.H.	37	1	+	+	±	+	+	±	Negative,	—
	A.E.B.H.	1	10	+	+	tr	+	+	±	Weakly positive,	1+
	A.I.B.H.	1	10	+	+	tr	+	+	±	Negative,	—
	A.E.B.H.	37	10	+	+	tr	+	+	tr	Negative,	—
	A.I.B.H.	37	10	+	+	tr	+	+	tr	Negative,	—
153	A.E.B.H.	1	1	+	+	±	+	+	±	Negative,	—
	A.I.B.H.	1	1	+	+	±	+	+	±	Negative,	—
	A.E.B.H.	37	1	+	+	±	+	+	±	Negative,	—
	A.I.B.H.	37	1	+	+	±	+	+	±	Negative,	—
	A.E.B.H.	1	10	+	+	tr	+	+	±	Weakly positive,	1+
	A.I.B.H.	1	10	+	+	tr	+	+	±	Negative,	—
	A.E.B.H.	37	10	+	+	tr	+	+	tr	Negative,	—
	A.I.B.H.	37	10	+	+	tr	+	+	tr	Negative,	—
154	A.E.B.H.	1	1	+	+	tr	+	+	±	Weakly positive,	1+
	A.I.B.H.	1	1	+	+	tr	+	+	±	Negative,	—
	A.E.B.H.	37	1	+	+	tr	+	+	±	Moderately positive,	2+
	A.I.B.H.	37	1	+	+	tr	+	+	±	Negative,	—
	A.E.B.H.	1	10	±	0	0	+	+	±	Strongly positive,	6+
	A.I.B.H.	1	10	+	+	tr	+	+	±	Negative,	—
	A.E.B.H.	37	10	+	+	tr	+	+	tr	Moderately positive,	2+
	A.I.B.H.	37	10	+	+	tr	+	+	tr	Weakly positive,	1+
155	A.E.B.H.	1	1	+	±	0	+	+	±	Strongly positive,	3+
	A.I.B.H.	1	1	+	+	±	+	+	±	Negative,	—
	A.E.B.H.	37	1	+	+	tr	+	+	±	Weakly positive,	1+
	A.I.B.H.	37	1	+	+	tr	+	+	±	Negative,	—
	A.E.B.H.	1	10	0	0	0	+	+	±	Strongly positive,	10+
	A.I.B.H.	1	10	+	+	±	+	+	±	Negative,	—
	A.E.B.H.	37	10	+	±	0	+	+	tr	Moderately positive,	2+
	A.I.B.H.	37	10	+	+	tr?	+	+	tr	Faintly positive,	±
156	A.E.B.H.	1	1	+	+	0	+	+	±	Moderately positive,	2+
	A.I.B.H.	1	1	+	+	±	+	+	±	Negative,	—
	A.E.B.H.	37	1	+	+	0	+	+	±	Moderately positive,	2+
	A.I.B.H.	37	1	+	+	±	+	+	±	Negative,	—
	A.E.B.H.	1	10	0	0	0	+	+	±	Strongly positive,	10+
	A.I.B.H.	1	10	+	+	±	+	+	±	Negative,	—
	A.E.B.H.	37	10	+	±	0	+	+	tr	Moderately positive,	2+
	A.I.B.H.	37	10	+	+	0	+	+	tr	Weakly positive,	1+

TABLE 7—Continued

ACETONE INSOLUBLE ANTIGEN COMPARED WITH CRUDE ALCOHOLIC EXTRACT, INCUBATION AT
1 C. AND 37 C. FOR 1 HOUR AND 10 HOURS

Number of Serum	Kind of Antigen	Incuba- tion Temper- ature, C.	Incuba- tion Time, Hours	Readings							Results	
				Antigen Tubes			Control Tubes					
				1	2	3	1'	2'	3'			
157 Dilution 1:5	A.E.B.H.	1	1	0	0	0	+	+	±	10+ × 5 = 50+		
	A.I.B.H.	1	1	+	±	0	+	+	±	3+ × 5 = 15+		
	A.E.B.H.	37	1	+	±	0	+	+	±	3+ × 5 = 15+		
	A.I.B.H.	37	1	+	+	tr	+	+	±	1+ × 5 = 5+		
	A.E.B.H.	1	10	0	0	0	+	+	±	10+ × 5 = 50+		
	A.I.B.H.	1	10	tr	0	0	+	+	±	8+ × 5 = 40+		
	A.E.B.H.	37	10	+	±	0	+	+	±	3+ × 5 = 15+		
	A.I.B.H.	37	10	+	+	0	+	+	±	2+ × 5 = 10+		
Dilution 1:25	A.E.B.H.	1	1	+	±	0	+	+	±	3+ × 25 = 75+		
	A.E.B.H.	1	1	+	+	±	+	+	±	Negative, —		
	A.E.B.H.	37	1	+	+	±	+	+	±	Negative, —		
	A.I.B.H.	37	1	+	+	±	+	+	±	Negative, —		
	A.E.B.H.	1	10	tr	0	0	+	+	±	8+ × 25 = 200+		
	A.I.B.H.	1	10	+	+	±	+	+	±	Negative, —		
	A.E.B.H.	37	10	+	+	tr	+	+	±	1+ × 25 = 25+		
	A.I.B.H.	37	10	+	+	tr	+	+	±	1+ × 25 = 25+		
157 (cont.) Dilution 1:125	A.E.B.H.	1	1	+	+	±	+	+	±	Negative, —		
	A.I.B.H.	1	1	+	+	±	+	+	±	Negative, —		
	A.E.B.H.	37	1	+	+	±	+	+	±	Negative, —		
	A.I.B.H.	37	1	+	+	±	+	+	±	Negative, —		
	A.E.B.H.	1	10	+	+	±	+	+	±	Negative, —		
	A.I.B.H.	1	10	+	+	±	+	+	±	Negative, —		
	A.E.B.H.	37	10	+	+	±	+	+	±	Negative, —		
	A.I.B.H.	37	10	+	+	±	+	+	±	Negative, —		
158 Dilution 1:5	A.E.B.H.	1	1	tr	0	0	+	+	±	8+ × 5 = 40+		
	A.I.B.H.	1	1	+	tr	0	0	+	±	4+ × 5 = 20+		
	A.E.B.H.	37	1	0	0	0	+	+	tr	8+ × 5 = 40+		
	A.I.B.H.	37	1	0	0	0	0	+	tr	8+ × 5 = 40+		
	A.E.B.H.	1	10	0	0	0	0	+	±	10+ × 5 = 50+		
	A.I.B.H.	1	10	tr	0	0	0	+	±	8+ × 5 = 40+		
	A.E.B.H.	37	10	0	0	0	+	+	tr	8+ × 5 = 40+		
	A.I.B.H.	37	10	0	0	0	+	+	tr	8+ × 5 = 40+		
Dilution 1:25	A.E.B.H.	1	1	+	+	0	+	+	±	2+ × 25 = 50+		
	A.I.B.H.	1	1	+	+	tr	+	+	±	1+ × 25 = 25+		
	A.E.B.H.	37	1	+	0	0	+	+	tr	4+ × 25 = 100+		
	A.I.B.H.	37	1	+	tr	0	0	+	tr	3+ × 25 = 75+		
	A.E.B.H.	1	10	tr	0	0	0	+	±	8+ × 25 = 200+		
	A.I.B.H.	1	10	+	+	tr	+	+	±	1+ × 25 = 25+		
	A.E.B.H.	37	10	+	0	0	+	+	tr	4+ × 25 = 100+		
	A.I.B.H.	37	10	+	0	0	+	+	tr	4+ × 25 = 100+		
Dilution 1:125	A.E.B.H.	1	1	+	+	±	+	+	±	Negative, —		
	A.I.B.H.	1	1	+	+	±	+	+	±	Negative, —		
	A.E.B.H.	37	1	+	+	0	+	+	tr	1+ × 125 = 125+		
	A.I.B.H.	37	1	+	+	tr	+	+	tr	Negative, —		
	A.E.B.H.	1	10	+	+	±	+	+	±	Negative, —		
	A.I.B.H.	1	10	+	+	±	+	+	±	Negative, —		
	A.E.B.H.	37	10	+	+	tr	+	+	tr	Negative, —		
	A.I.B.H.	37	10	+	+	tr	+	+	tr	Negative, —		
159 Dilution 1:5	A.E.B.H.	1	1	tr	0	0	+	+	tr	6+ × 5 = 30+		
	A.I.B.H.	1	1	tr	0	0	+	+	tr	6+ × 5 = 30+		
	A.E.B.H.	37	1	0	0	0	0	+	0	6+ × 5 = 30+		
	A.I.B.H.	37	1	0	0	0	0	+	0	6+ × 5 = 30+		
	A.E.B.H.	1	10	0	0	0	0	+	0	6+ × 5 = 30+		
	A.I.B.H.	1	10	0	0	0	0	+	0	6+ × 5 = 30+		
	A.E.B.H.	37	10	0	0	0	0	+	0	3+ × 5 = 15+		
	A.I.B.H.	37	10	0	0	0	0	+	0	3+ × 5 = 15+		

TABLE 7—Continued

ACETONE INSOLUBLE ANTIGEN COMPARED WITH CRUDE ALCOHOLIC EXTRACT, INCUBATION AT 1 C. AND 37 C. FOR 1 HOUR AND 10 HOURS

Number of Serum	Kind of Antigen	Incuba- tion Temper- ature, C.	Incuba- tion Time, Hours	Readings							Results	
				Antigen Tubes			Control Tubes					
				1	2	3	1'	2'	3'			
Dilution 1:25	A.E.B.H.	1	1	+	tr	0	+	+	±	4+ × 25 = 100+		
	A.I.B.H.	1	1	+	+	±	+	+	±	Negative, —		
	A.E.B.H.	37	1	±	0	0	+	+	tr	5+ × 25 = 125+		
	A.I.B.H.	37	1	tr	0	0	+	+	tr	6+ × 25 = 150+		
	A.E.B.H.	1	10	0	0	0	+	+	tr	8+ × 25 = 200+		
	A.I.B.H.	1	10	+	±	0	+	+	tr	2+ × 25 = 50+		
	A.E.B.H.	37	10	0	0	0	+	+	0	6+ × 25 = 150+		
	A.I.B.H.	37	10	0	0	0	+	+	0	6+ × 25 = 150+		
Dilution 1:125	A.E.B.H.	1	1	+	+	tr	+	+	±	1+ × 125 = 125+		
	A.I.B.H.	1	1	+	+	±	+	+	±	Negative, —		
	A.E.B.H.	37	1	+	±	0	+	+	tr	2+ × 125 = 250+		
	A.I.B.H.	37	1	+	+	0	+	+	tr	1+ × 125 = 125+		
	A.E.B.H.	1	10	+	0	0	+	+	±	5+ × 125 = 625+		
	A.I.B.H.	1	10	+	+	tr	+	+	±	1+ × 125 = 125+		
	A.E.B.H.	37	10	+	±	0	+	+	tr	2+ × 125 = 250+		
	A.I.B.H.	37	10	+	±	0	+	+	tr	2+ × 125 = 250+		
Dilution 1:10	A.E.B.H.	1	1	0	0	0	+	+	±	10+ × 10 = 100+		
	A.I.B.H.	1	1	+	0	0	+	+	±	5+ × 10 = 50+		
	A.E.B.H.	37	1	0	0	0	+	+	tr	8+ × 10 = 80+		
	A.I.B.H.	37	1	0	0	0	+	+	tr	8+ × 10 = 80+		
	A.E.B.H.	1	10	0	0	0	+	+	tr	8+ × 10 = 80+		
	A.I.B.H.	1	10	0	0	0	+	+	tr	8+ × 10 = 80+		
	A.E.B.H.	37	10	0	0	0	+	+	0	6+ × 10 = 60+		
	A.I.B.H.	37	10	0	0	0	+	+	0	6+ × 10 = 60+		
Dilution 1:50	A.E.B.H.	1	1	+	±	0	+	+	±	3+ × 50 = 150+		
	A.I.B.H.	1	1	+	+	tr	+	+	±	1+ = 50 = 50+		
	A.E.B.H.	37	1	+	0	0	+	+	tr	4+ × 50 = 200+		
	A.I.B.H.	37	1	+	+	0	+	+	tr	1+ × 50 = 50+		
	A.E.B.H.	1	10	0	0	0	+	+	±	10+ × 50 = 500+		
	A.I.B.H.	1	10	+	0	0	+	+	tr	2+ × 50 = 100+		
	A.E.B.H.	37	10	+	0	0	+	+	tr	4+ × 50 = 200+		
	A.I.B.H.	37	10	+	±	0	+	+	tr	2+ × 50 = 100+		
Dilution 1:250	A.E.B.H.	1	1	+	+	±	+	+	±	Negative, —		
	A.I.B.H.	1	1	+	+	±	+	+	±	Negative, —		
	A.E.B.H.	37	1	+	+	±	+	+	±	Negative, —		
	A.I.B.H.	37	1	+	+	±	+	+	±	Negative, —		
	A.E.B.H.	1	10	+	+	0	+	+	±	2+ × 250 = 500+		
	A.I.B.H.	1	10	+	0	±	+	+	±	Negative, —		
	A.E.B.H.	37	10	+	+	tr	+	+	tr	Negative, —		
	A.I.B.H.	37	10	+	+	tr	+	+	tr	Negative, —		

Table 7 shows the results obtained by comparing acetone insoluble antigen of beef heart with alcoholic extract of beef heart. Alcoholic extract of beef heart gave much stronger positive results than did the acetone insoluble antigen, and incubation at 1 C. gave much stronger positive results than did incubation at 37 C. With the acetone insoluble antigen incubation at 37 C. gave stronger positive results than did incubation at 1 C.

TEST 8

Alcoholic extract of beef heart (A. E. B. H.) was compared with alcoholic extract of human heart (A. E. H. H.) at 1 C. and 37 C. for 1 hour and 10 hours on 12 serums—161 to 172, inclusive.

TABLE 8
ALCOHOLIC EXTRACT OF BEEF HEART COMPARED WITH ALCOHOLIC EXTRACT OF HUMAN HEART,
INCUBATION AT 1 C. AND 37 C. FOR 1 HOUR AND 10 HOURS

Number of Serum	Kind of Antigen	Incuba- tion Temper- ature, C.	Incuba- tion Time, Hours	Readings						Results	
				Antigen Tubes			Control Tubes				
				1	2	3	1'	2'	3'		
161	A.E.H.H.	1	1	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.B.H.	1	1	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.H.H.	37	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.B.H.	37	1	+	0	0	+	+	±	Moderately positive, 2+	
	A.E.H.H.	1	10	+	0	0	+	+	±	Strongly positive, 5+	
	A.E.B.H.	1	10	+	0	0	+	+	±	Strongly positive, 5+	
	A.E.H.H.	37	10	+	±	0	+	+	tr	Moderately positive, 2+	
	A.E.B.H.	37	10	+	±	0	+	+	tr	Moderately positive, 2+	
162	A.E.H.H.	1	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.B.H.	1	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.H.H.	37	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.B.H.	37	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.H.H.	1	10	0	0	0	+	+	±	Strongly positive, 10+	
	A.E.B.H.	1	10	0	0	0	+	+	±	Strongly positive, 10+	
	A.E.H.H.	37	10	+	±	0	+	+	tr	Moderately positive, 2+	
	A.E.B.H.	37	10	+	±	0	+	+	tr	Moderately positive, 2+	
163	A.E.H.H.	1	1	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.B.H.	1	1	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.H.H.	37	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.B.H.	37	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.H.H.	1	10	0	0	0	+	+	±	Strongly positive, 10+	
	A.E.B.H.	1	10	0	0	0	+	+	±	Strongly positive, 10+	
	A.E.H.H.	37	10	+	±	0	+	+	tr	Moderately positive, 2+	
	A.E.B.H.	37	10	+	±	0	+	+	tr	Moderately positive, 2+	
164	A.E.H.H.	1	1	+	0	0	+	+	±	Moderately positive, 2+	
	A.E.B.H.	1	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.H.H.	37	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.B.H.	37	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.H.H.	1	10	0	0	0	+	+	±	Strongly positive, 10+	
	A.E.B.H.	1	10	0	0	0	+	+	±	Strongly positive, 10+	
	A.E.H.H.	37	10	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.B.H.	37	10	+	+	0	+	+	±	Moderately positive, 2+	
165	A.E.H.H.	1	1	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.B.H.	1	1	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.H.H.	37	1	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.B.H.	37	1	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.H.H.	1	10	+	0	0	+	+	±	Strongly positive, 5+	
	A.E.B.H.	1	10	+	0	0	+	+	±	Strongly positive, 5+	
	A.E.H.H.	37	10	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.B.H.	37	10	+	+	tr	+	+	±	Weakly positive, 1+	
166	A.E.H.H.	1	1	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.B.H.	1	1	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.H.H.	37	1	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.B.H.	37	1	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.H.H.	1	10	tr	0	0	+	+	±	Strongly positive, 8+	
	A.E.B.H.	1	10	±	0	0	+	+	±	Strongly positive, 6+	
	A.E.H.H.	37	10	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.B.H.	37	10	+	+	tr	+	+	±	Weakly positive, 1+	

TABLE 8—Continued

ALCOHOLIC EXTRACT OF BEEF HEART COMPARED WITH ALCOHOLIC EXTRACT OF HUMAN HEART,
INCUBATION AT 1 C. AND 37 C. FOR 1 HOUR AND 10 HOURS

Number of Serum	Kind of Antigen	Incuba- tion Temper- ature, C.	Incuba- tion Time, Hours	Readings						Results	
				Antigen Tubes			Control Tubes				
				1	2	3	1'	2'	3'		
167	A.E.H.H.	1	1	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.B.H.	1	1	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.H.H.	37	1	+	+	±?	+	+	±	Faintly positive, ±	
	A.E.B.H.	37	1	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.H.H.	1	10	0	0	0	0	0	+	Strongly positive, 10+	
	A.E.B.H.	1	10	0	0	0	+	+	±	Strongly positive, 10+	
	A.E.H.H.	37	10	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.B.H.	37	10	+	+	0	+	+	±	Moderately positive, 2+	
168	A.E.H.H.	1	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.B.H.	1	1	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.H.H.	37	1	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.B.H.	37	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.H.H.	1	10	tr	0	0	+	+	±	Strongly positive, 8+	
	A.E.B.H.	1	10	tr	0	0	+	+	±	Strongly positive, 8+	
	A.E.H.H.	37	10	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.B.H.	37	10	+	+	0	+	+	±	Moderately positive, 2+	
169	A.E.H.H.	1	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.B.H.	1	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.H.H.	37	1	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.B.H.	37	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.H.H.	1	10	0	0	0	0	0	+	Strongly positive, 10+	
	A.E.B.H.	1	10	0	0	0	+	+	±	Strongly positive, 10+	
	A.E.H.H.	37	10	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.B.H.	37	10	+	+	0	+	+	±	Moderately positive, 2+	
170	A.E.H.H.	1	1	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.B.H.	1	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.H.H.	37	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.B.H.	37	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.H.H.	1	10	0	0	0	+	+	±	Strongly positive, 10+	
	A.E.B.H.	1	10	0	0	0	+	+	±	Strongly positive, 10+	
	A.E.H.H.	37	10	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.B.H.	37	10	+	+	0	+	+	±	Moderately positive, 2+	
171	A.E.H.H.	1	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.B.H.	1	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.H.H.	37	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.B.H.	37	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.H.H.	1	10	0	0	0	+	+	±	Strongly positive, 10+	
	A.E.B.H.	1	10	0	0	0	+	+	±	Strongly positive, 10+	
	A.E.H.H.	37	10	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.B.H.	37	10	+	+	tr	0	+	tr	Strongly positive, 3+	
172	A.E.H.H.	1	1	+	+	tr	+	+	±	Weakly positive, 1+	
	A.E.B.H.	1	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.H.H.	37	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.B.H.	37	1	+	+	0	+	+	±	Moderately positive, 2+	
	A.E.H.H.	1	10	tr	0	0	+	+	±	Strongly positive, 8+	
	A.E.B.H.	1	10	0	0	0	+	+	±	Strongly positive, 10+	
	A.E.H.H.	37	10	+	±	0	+	+	tr	Moderately positive, 2+	
	A.E.B.H.	37	10	+	±	0	+	+	tr	Moderately positive, 2+	

Table 8 shows that with very slight exceptions the results obtained with alcoholic extract of beef heart were identical with the results obtained with alcoholic extract of human heart. Slight differences are noticed with serums 166, 167, 168, 169, 171 and 172. These differences are so slight that they may be due to technical errors.

TEST 9

With Serums 173 to 184, inclusive, acetone insoluble antigen of beef heart (A. I. B. H.) was compared with alcoholic extract of human heart (A. E. H. H.) at 1 C. and 37 C. for 1 hour and 10 hours.

TABLE 9
ACETONE INSOLUBLE ANTIGEN OF BEEF HEART COMPARED WITH ALCOHOLIC EXTRACT OF HUMAN
HEART, INCUBATION AT 1 C. AND 37 C. FOR 1 HOUR AND 10 HOURS

Number of Serum	Kind of Antigen	Incuba- tion Tempera- ture, C.	Incuba- tion Time, Hours	Readings						Results	
				Antigen Tubes			Control Tubes				
				1	2	3	1'	2'	3'		
173	A.E.H.H.	1	1	+	+	±	+	+	±	Negative,	—
	A.I.B.H.	1	1	+	+	±	+	+	±	Negative,	—
	A.E.H.H.	37	1	+	+	tr	+	+	±	Weakly positive,	1+
	A.I.B.H.	37	1	+	+	±	+	+	±	Negative,	—
	A.E.H.H.	1	10	+	0	0	+	+	±	Strongly positive,	5+
	A.I.B.H.	1	10	+	+	±	+	+	±	Negative,	—
	A.E.H.H.	37	10	+	+	0	+	+	±	Moderately positive,	2+
	A.I.B.H.	37	10	+	+	tr	+	+	±	Weakly positive,	1+
174	A.E.H.H.	1	1	+	+	±?	+	+	±	Faintly positive,	±
	A.I.B.H.	1	1	+	+	±	+	+	±	Negative,	—
	A.E.H.H.	37	1	+	+	±?	+	+	±	Faintly positive,	±
	A.I.B.H.	37	1	+	+	±	+	+	±	Negative,	—
	A.E.H.H.	1	10	tr	0	0	+	+	±	Strongly positive,	8+
	A.I.B.H.	1	10	+	+	±	+	+	±	Negative,	—
	A.E.H.H.	37	10	+	+	tr	+	+	±	Weakly positive,	1+
	A.I.B.H.	37	10	+	+	±	+	+	±	Negative,	—
175	A.E.H.H.	1	1	+	+	±	+	+	±	Negative,	—
	A.I.B.H.	1	1	+	+	±	+	+	±	Negative,	—
	A.E.H.H.	37	1	+	+	±	+	+	±	Negative,	—
	A.I.B.H.	37	1	+	+	±	+	+	±	Negative,	—
	A.E.H.H.	1	10	tr	0	0	+	+	±	Strongly positive,	4+
	A.I.B.H.	1	10	+	+	±	+	+	±	Negative,	—
	A.E.H.H.	37	10	+	+	tr	+	+	±	Weakly positive,	1+
	A.I.B.H.	37	10	+	+	±?	+	+	±	Faintly positive,	±
176	A.E.H.H.	1	1	+	tr	0	+	+	±	Strongly positive,	4+
	A.I.B.H.	1	1	+	+	±	+	+	±	Negative,	—
	A.E.H.H.	37	1	+	±	0	+	+	±	Strongly positive,	3+
	A.I.B.H.	37	1	+	+	tr	+	+	±	Weakly positive,	1+
	A.E.H.H.	1	10	0	0	0	+	+	±	Strongly positive,	10+
	A.I.B.H.	1	10	+	+	±	+	+	±	Negative,	—
	A.E.H.H.	37	10	+	±	0	+	+	±	Strongly positive,	3+
	A.I.B.H.	37	10	+	+	tr	+	+	±	Weakly positive,	1+
177	A.E.H.H.	1	1	+	±	0	+	+	±	Strongly positive,	3+
	A.I.B.H.	1	1	+	+	±	+	+	±	Negative,	—
	A.E.H.H.	37	1	+	+	±	0	+	±	Moderately positive,	2+
	A.I.B.H.	37	1	+	+	±?	0	+	±	Faintly positive,	±
	A.E.H.H.	1	10	0	0	0	+	+	±	Strongly positive,	10+
	A.I.B.H.	1	10	+	+	±	0	+	±	Negative,	—
	A.E.H.H.	37	10	+	+	0	+	+	±	Moderately positive,	2+
	A.I.B.H.	37	10	+	+	tr	+	+	±	Weakly positive,	1+
178	A.E.H.H.	1	1	+	±	0	+	+	±	Strongly positive,	3+
	A.I.B.H.	1	1	+	+	±	0	+	±	Negative,	—
	A.E.H.H.	37	1	+	+	0	+	+	±	Moderately positive,	2+
	A.I.B.H.	37	1	+	+	±?	0	+	±	Faintly positive,	±
	A.E.H.H.	1	10	0	0	0	+	+	±	Strongly positive,	10+
	A.I.B.H.	1	10	+	+	±	0	+	±	Negative,	—
	A.E.H.H.	37	10	+	+	0	+	+	±	Moderately positive,	2+
	A.I.B.H.	37	10	+	+	±?	0	+	±	Faintly positive,	±

TABLE 9—Continued

ACETONE INSOLUBLE ANTIGEN OF BEEF HEART COMPARED WITH ALCOHOLIC EXTRACT OF HUMAN HEART, INCUBATION AT 1 C. AND 37 C. FOR 1 HOUR AND 10 HOURS

Number of Serum	Kind of Antigen	Incuba- tion Temper- ature, C.	Incuba- tion Time, Hours	Readings							Results	
				Antigen Tubes			Control Tubes					
				1	2	3	1'	2'	3'			
179	A.E.H.H.	1	1	+	tr	0	+	+	+	±	Strongly positive, 4+	
	A.I.B.H.	1	1	+	+	±	+	+	+	±	Negative, —	
	A.E.H.H.	37	1	+	tr	0	+	+	+	±	Strongly positive, 4+	
	A.I.B.H.	37	1	+	+	0	+	+	+	±	Moderately positive, 2+	
	A.E.H.H.	1	10	0	0	0	+	+	+	±	Strongly positive, 10+	
	A.I.B.H.	1	10	+	+	±	+	+	+	±	Negative, —	
	A.E.H.H.	37	10	+	tr	0	+	+	+	±	Strongly positive, 4+	
	A.I.B.H.	37	10	+	+	0	+	+	+	±	Moderately positive, 2+	
180	A.E.H.H.	1	1	+	tr	0	+	+	+	±	Strongly positive, 4+	
	A.I.B.H.	1	1	+	+	±	+	+	+	±	Negative, —	
	A.E.H.H.	37	1	+	+	0	+	+	+	±	Moderately positive, 2+	
	A.I.B.H.	37	1	+	+	tr	+	+	+	±	Weakly positive, 1+	
	A.E.H.H.	1	10	0	0	0	+	+	+	±	Strongly positive, 10+	
	A.I.B.H.	1	10	+	+	±	+	+	+	±	Negative, —	
	A.E.H.H.	37	10	+	+	0	+	+	+	±	Moderately positive, 2+	
	A.I.B.H.	37	10	+	+	tr	+	+	+	±	Weakly positive, 1+	
181	A.E.H.H.	1	1	+	tr	0	+	+	+	±	Strongly positive, 4+	
	A.I.B.H.	1	1	+	+	±	+	+	+	±	Negative, —	
	A.E.H.H.	37	1	+	tr	0	+	+	+	±	Strongly positive, 4+	
	A.I.B.H.	37	1	+	+	0	+	+	+	±	Moderately positive, 2+	
	A.E.H.H.	1	10	0	0	0	+	+	+	±	Strongly positive, 10+	
	A.I.B.H.	1	10	+	+	±	+	+	+	±	Negative, —	
	A.E.H.H.	37	10	+	tr	0	+	+	+	±	Strongly positive, 4+	
	A.I.B.H.	37	10	+	+	0	+	+	+	±	Moderately positive, 2+	
182	A.E.H.H.	1	1	+	tr	0	+	+	+	±	Strongly positive, 4+	
	A.I.B.H.	1	1	+	+	±	+	+	+	±	Negative, —	
	A.E.H.H.	37	1	+	+	0	+	+	+	±	Moderately positive, 2+	
	A.I.B.H.	37	1	+	+	tr	+	+	+	±	Weakly positive, 1+	
	A.E.H.H.	1	10	0	0	0	+	+	+	±	Strongly positive, 10+	
	A.I.B.H.	1	10	+	+	±	+	+	+	±	Negative, —	
	A.E.H.H.	37	10	+	+	0	+	+	+	±	Moderately positive, 2+	
	A.I.B.H.	37	10	+	+	0	+	+	+	±	Moderately positive, 2+	
183	A.E.H.H.	1	1	+	tr	0	+	+	+	±	Strongly positive, 4+	
	A.I.B.H.	1	1	+	+	±	+	+	+	±	Negative, —	
	A.E.H.H.	37	1	+	+	0	+	+	+	±	Moderately positive, 2+	
	A.I.B.H.	37	1	+	+	tr	+	+	+	±	Weakly positive, 1+	
	A.E.H.H.	1	10	0	0	0	+	+	+	±	Strongly positive, 10+	
	A.I.B.H.	1	10	+	+	±	+	+	+	±	Negative, —	
	A.E.H.H.	37	10	+	+	0	+	+	+	±	Strongly positive, 3+	
	A.I.B.H.	37	10	+	+	0	+	+	+	±	Moderately positive, 2+	
184	A.E.H.H.	1	1	+	tr	0	+	+	+	±	Strongly positive, 4+	
	A.I.B.H.	1	1	+	+	±	+	+	+	±	Negative, —	
	A.E.H.H.	37	1	+	+	0	+	+	+	±	Moderately positive, 2+	
	A.I.B.H.	37	1	+	+	0	+	+	+	±	Moderately positive, 2+	
	A.E.H.H.	1	10	0	0	0	+	+	+	±	Strongly positive, 10+	
	A.I.B.H.	1	10	+	+	±	+	+	+	±	Negative, —	
	A.E.H.H.	37	10	+	+	0	+	+	+	±	Strongly positive, 3+	
	A.I.B.H.	37	10	+	+	0	+	+	+	±	Moderately positive, 2+	

Table 9 shows that alcoholic extract of human heart gave much stronger positive results than did acetone insoluble antigen of beef heart. With alcoholic extract of human heart incubation at 1 C. gave much stronger positive results than did incubation at 37 C., while with acetone insoluble antigen stronger positive results were obtained at 37 C. than at 1 C.

TEST 10

When sensitized blood corpuscles are added to serum-complement-antigen mixture immediately after the latter has been removed from the water-bath at 1 C. and the whole mixture then placed in the incubator at 37 C. hemolysis does not take place until the contents of the tubes have become warm, while agglutination of the corpuscles is rapid even at a temperature of 1 C. In order to determine whether or not the mixture may be warmed before the sensitized blood corpuscles are added each of 16 serums (185 to 200, inclusive) was divided into two portions, A and B. Portion A was left in the water-bath at 1 C. for 10 hours and the sensitized blood corpuscles were added immediately after the tubes had been removed from the cold water-bath. Portion B was left in the cold water-bath for 9.5 hours, then was placed in the incubator at 37 C. for 0.5 hour before the sensitized blood corpuscles were added.

TABLE 10
NINE AND ONE-HALF HOUR INCUBATION AT 1 C. + ONE-HALF HOUR AT 37 C. COMPARED WITH
TEN-HOUR INCUBATION AT 1 C.

Number of Serum	Portion	Incuba- tion Temper- ature, C.	Incuba- tion Time, Hours	Readings						Results	
				Antigen Tubes			Control Tubes				
				1	2	3	1'	2'	3'		
185	A	1	10	+	tr	0	+	+	±	Strongly positive,	4+
	B	1	9.5	+	tr	0	+	+	±	Strongly positive,	4+
186	A	1	10	±	0	0	+	+	±	Strongly positive,	6+
	B	1	9.5	±	0	0	+	+	±	Strongly positive,	6+
187	A	1	10	tr	0	0	+	+	±	Strongly positive,	8+
	B	1	9.5	tr	0	0	+	+	±	Strongly positive,	8+
188	A	1	10	±	0	0	+	+	±	Strongly positive,	6+
	B	1	9.5	±	0	0	+	+	±	Strongly positive,	6+
189	A	1	10	+	tr	0	+	+	±	Strongly positive,	4+
	B	1	9.5	+	tr	0	+	+	±	Strongly positive,	4+
190	A	1	10	+	0	0	+	+	±	Strongly positive,	5+
	B	1	9.5	+	0	0	+	+	±	Strongly positive,	5+
191	A	1	10	±	0	0	+	+	±	Strongly positive,	6+
	B	1	9.5	+	0	0	+	+	±	Strongly positive,	5+
192	A	1	10	+	tr	0	+	+	±	Strongly positive,	4+
	B	1	9.5	+	tr	0	+	+	±	Strongly positive,	4+
193	A	1	10	±	0	0	+	+	±	Strongly positive,	6+
	B	1	9.5	±	0	0	+	+	±	Strongly positive,	6+
194	A	1	10	+	±	0	+	+	±	Strongly positive,	3+
	B	1	9.5	+	±	0	+	+	±	Strongly positive,	3+
195	A	1	10	+	+	tr	+	+	±	Weakly positive,	1+
	B	1	9.5	+	+	tr	+	+	±	Weakly positive,	1+
196	A	1	10	+	+	0	+	+	±	Moderately positive,	2+
	B	1	9.5	+	+	0	+	+	±	Moderately positive,	2+
197	A	1	10	+	±	0	+	+	±	Strongly positive,	3+
	B	1	9.5	+	±	0	+	+	±	Strongly positive,	3+
198	A	1	10	+	0	0	+	+	±	Strongly positive,	5+
	B	1	9.5	+	0	0	+	+	±	Strongly positive,	5+
199	A	1	10	+	±	0	+	+	±	Strongly positive,	3+
	B	1	9.5	+	±	0	+	+	±	Strongly positive,	8+
200	A	1	10	+	tr	0	+	+	±	Strongly positive,	4+
	B	1	9.5	+	tr	0	+	+	±	Strongly positive,	4+

Table 10 shows that portion B gave results that were identical with those given by portion A. In portion A the corpuscles were badly agglutinated before hemolysis took place; the tubes had to be frequently shaken and hemolysis was very slow, while in portion B hemolysis was rapid and with little or no agglutination of the blood corpuscles.

SUMMARY AND CONCLUSIONS

Incubation at 1 C. gave stronger positive results than did incubation at 2 C., 3 C., 4 C., 5 C., 6 C., 7 C., 8 C., 9 C., 10 C., or 37 C., while the results obtained at 0.5 C. were identical with those obtained at 1 C.

Incubation for 24 hours at 1 C. gave stronger positive results than incubation at 1 C. for 15 hours; incubation for 15 hours at 1 C. gave stronger positive results than did incubation for 10 hours at 1 C.; incubation for 10 hours at 1 C. gave stronger positive results than did incubation for 5 hours at 1 C., and incubation for 5 hours at 1 C. gave stronger positive results than did incubation for 1 hour at 1 C.

Incubation for 10 hours at 1 C. gave uniformly good results, while with one lot of complement 15-hour incubation at 1 C. and 24-hour incubation at 1 C. gave bad results. At the present time 10-hour incubation at 1 C. is the longest incubation time which has met all requirements.

Twenty-four serums from healthy persons gave negative results under 10-hour incubation at 1 C. and under 15-hour incubation at 1 C.

Alcoholic extract of beef heart gave much stronger positive results than did acetone insoluble antigen of beef heart. The alcoholic extract gave the strong positive results at 1 C., while the acetone insoluble antigen gave stronger positive results at 37 C. than at 1 C.

At 1 C. alcoholic extract of beef heart gave results that were identical with the results given by alcoholic extract of human heart at 1 C.

Acetone insoluble antigen of beef heart gave much weaker positive results than did alcoholic extract of human heart.

Warming the serum-complement-antigen mixture before the sensitized blood corpuscles were added gave the same results as when the sensitized blood corpuscles were added to the cold serum-complement-antigen mixture. Warming the serum-complement-antigen mixture before the sensitized blood corpuscles are added is a decided advantage because hemolysis is much more rapid and agglutination of the corpuscles is much less.